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REVIEW

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Urinary cotinine as a tobacco-smoke exposure index: a minireview

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Abstract A minireview is presented concerning the use of cotinine as a tobacco-smoke exposure index. First, general considerations about methods for the determination of urinary cotinine are presented. Besides pure analytical aspects, this minireview considers major problems encountered in the establishment of threshold values that can be used to distinguish not only smokers from nonsmokers but also nonsmokers exposed or not exposed to environmental tobacco smoke (ETS). In addition, the use of urinary cotinine is illustrated in several situations where smoking status assessment is of interest. Such situations include evaluation of the impact of smoking cessation programs, monitoring of pregnancy and of other groups at risk, assessment of occupational exposure to industrial pollutants, validation of phase I clinical trials, and the control of life insurance candidates. The specific problem of ETS exposure assessment is briefly mentioned.

Key words Biomarkers · Cotinine · Smoking

Introduction

Several biological markers have been proposed for assessment of the importance of direct or passive exposure to tobacco smoke. The most widely used markers are carboxyhemoglobin in blood; carbon monoxide in expired air; thiocyanate and nicotine in saliva, plasma, or urine; and cotinine, a major nicotine metabolite, which can be determined not only in urine but also in plasma and saliva [38]. Some markers may be influenced by environmental sources other than tobacco smoke, namely, diet for thiocyanate and road traffic or domestic emissions for carbon monoxide and carboxyhemoglobin. Therefore, these markers have been progressively replaced by nicotine and cotinine (Fig. 1), which are more specific for tobacco smoke exposure. Because of the longer urinary half-life of cotinine as compared with nicotine (19 versus 2 h) [5, 102] and of the absence of ambient contamination during sample acquisition (the unique source of cotinine is nicotine metabolization), cotinine is currently considered the marker of choice. Cotinine is generally determined in urine, a biological medium easy to obtain, especially in the framework of epidemiological studies. This minireview considers the value of urinary cotinine determination as a tobacco-smoke exposure index and summarizes its main applications essentially related to active smoking screening. A review on the use of cotinine as a biomarker of environmental tobacco smoke (ETS) exposure has been published recently [4].

Analytical aspects

The *colorimetric method* is the simplest and cheapest method for urinary cotinine determination. Since it quantifies the totality of metabolites for which the pyridine ring remains intact, this method has low specificity. Both cotinine and *trans*-3′-hydroxycotinine (Fig. 1; the main metabolite formed from cotinine [12, 37, 63]) are measured. The results, expressed as cotinine equivalents, are therefore higher than those obtained by more specific methods such as gas or liquid chromatography [41]. However, the measured levels are well correlated with daily cigarette consumption [10, 41]. This method must therefore be considered a reliable index of smoking status, provided that blanks are included to take into account the coloration of each urine sample [41, 94]. However, interferences due, for example, to the presence of drugs with a pyridine ring (nicotinic acid, isoniazid, nicotinamide) are important [70, 94]. Moreover, the high detection threshold makes

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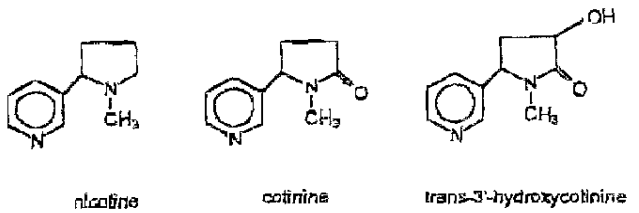

Fig. 1 Structures of nicotine, cotinine, and *trans*-3'-hydroxycotinine

this method inappropriate for monitoring of ETS exposure (see below) [41].

The *immunological methods* use monoclonal [45] or polyclonal antibodies that specifically recognize cotinine; they normally show only minimal cross-reactivity with nicotine and other metabolites [97]. However, a study has shown that *trans*-3'-hydroxycotinine cross-reacts by about 30% with the polyclonal rabbit anti-cotinine antiserum commonly used to determine cotinine levels by enzyme-linked immunosorbent assay (ELISA) [82], which does not seem to be the case for monoclonal antibodies [44]. Besides this possible cross-reactivity, immunoassays require the use of costly reagents [70]. The principal advantages of immunological methods are low sample-volume requirements, little sample manipulation, and rapidity of analysis [97]. The detection threshold of such immunoassays is also sufficient for ETS exposure assessment.

The *chromatography methods* high-performance liquid chromatography [3, 32, 33, 41, 59, 67, 77, 78, 94, 103] and gas chromatography [26] are most specific, especially when the identity of the peaks can be verified by mass spectrometry [18, 36, 51, 53, 89], but this requires the use of costly material and the resource of skilled personnel for routine analysis. Another advantage of chromatography methods is the simultaneous quantification of nicotine and cotinine in a single analysis [97]. The reagent costs are also generally low and the limits of detection are low, which makes these methods very suitable for passive smoking studies.

Value of urinary cotinine determination for active and passive smoking screening

General considerations

Active smoking of smokers voluntary exposed to tobacco smoke must be clearly distinguished from passive smoking of nonsmokers exposed to ETS produced by active smokers.

Whatever the determination method of urinary cotinine may be, rather good correlations exist between urinary cotinine and daily tobacco consumption (e.g., expressed in grams). Correlation coefficients mentioned in several studies vary from 0.39 to 0.99 but are generally greater than 0.75 [10, 28, 29, 41, 91, 95, 96, 101]. However, a major difficulty in establishing such correlation must be pointed out: urinary cotinine determination gives only information about recent exposure to tobacco smoke. Therefore, tobacco consumption during the 48-h period before urinary sampling must be representative of the announced daily consumption. This correlation theoretically allows the determination of a threshold value beyond which subjects can be classified as smokers. Despite rather high levels of passive exposure in some cases, several studies show that urinary cotinine levels in nonsmokers are always less than 100 ng/ml urine [6, 41]. In agreement with these data, discrimination thresholds between active and passive smokers vary, according to the authors, from 20 to 100 ng/ml urine or mg creatinine [38, 46, 50, 57, 58, 60, 69, 75, 76, 80, 81, 91].

Curiously, higher thresholds of 500 [1, 25] and 1000 ng/ml urine or mg creatinine [48] have been proposed when urinary determination is based on a fluorescence polarization immuno-assay (FPIA). However, these authors indicate that cotinine levels are lower than 80 ng/ml urine in the majority of nonsmokers [25] or that a threshold value of 50 ng/ml urine would have been more appropriate [1].

Although such thresholds can be used to distinguish smokers from nonsmokers, it is more difficult to quantify passive exposure to tobacco smoke. The first difficulty results from the very low urinary cotinine concentrations associated with passive smoking. When one uses a method measuring nicotine and cotinine at the same time, low quantities of nicotine possibly present in ambient air may contaminate the urinary samples in different handling steps [42]. Concerning the possible interference of nicotine in air, it must be stressed that when other ETS substances can no longer be quantified, nicotine may yet be measurable in ambient air [4]. Moreover, variations between laboratories are important because measured levels are close to analytical detection limits [6].

The influence of the age of exposed subjects constitutes the second problem. At the same level of ETS exposure, young children have higher urinary cotinine levels than adults [102]. Differences in the body distribution and metabolization of nicotine between adults and children have been mentioned [84]. A higher relative ventilation rate in young children could also explain such differences [102]. Some studies have shown that breast feeding is related to higher urinary cotinine excretion in children with smoking mothers [14, 19, 79] and that among these children, urinary cotinine excretion may reach the range observed in adult smokers [84]. Moreover, a recent study suggests racial differences among children; at the same ETS exposure level, black children have higher urinary cotinine levels than white children [40].

Finally, the presence of nicotine in some foods has been demonstrated and their consumption could be a source of urinary cotinine excretion. Among these foods are common dietary constituents such as eggplants, potatoes, and tomatoes (Solanaceae) as well as some tea plants. Considering the average and maximal daily

consumption of these foods, urinary cotinine values of 0.6 and 6.2 ng/ml, respectively, have been calculated [20]. Practically, however, these interferences remain negligible except in the case of vegetarians [34] but it should be stressed that not all dietary or other nontobacco sources of cotinine have been studied, which necessitates some caution [23].

All these factors are therefore potential explanations for differences observed in the following proposed threshold values between nonsmokers exposed or not exposed to ETS: 5 ng/ml urine [83], 10 ng/ml urine [14, 16], and 10 ng/mg creatinine [2, 50]. In agreement with the higher excretion rate observed in children, a higher threshold value of 30 ng cotinine/mg creatinine has been proposed in a study on children exposed to ETS [24].

Particular applications

Monitoring of ETS exposure in children/adults

Several studies have considered the impact of parental smoking habits on tobacco smoke exposure of children and possible health repercussions. They show a strongly positive correlation between urinary cotinine in children and the smoking habits of their parents [21, 27, 40, 73, 101]. When only one of the parents smokes, urinary cotinine is higher when the mother smokes [8, 14, 47, 83, 101]. Assessment of ETS exposure is particularly interesting in children with asthma, for whom it has been shown that passive smoking may be not only a source of asthma attack [16, 24, 100] but also considered an important aggravation factor of the disease [61]. To the best of our knowledge, only one study did not show an alteration of bronchial responsiveness in children with mild bronchial asthma after passive exposure to tobacco smoke, but the exposure duration was low (1 h at the equivalent of about 20 ppm CO) [65]. Children with or without a medical history of allergy (e.g., asthma) who are exposed to ETS have a higher incidence of respiratory infection [2, 73, 79, 84] and lower levels of respiratory performance [13] than do nonexposed children. These data illustrate the necessity of the appropriate information of parents and the establishment of intervention programs [15, 30]. Though less frequent, similar studies have considered the effects of passive smoking on healthy adults [42]. Moreover, the carcinogenesis risk has been tested [22, 74]. However, difficulties in obtaining statistically significant associations on the small scale sometimes require the realization of meta-analyses [93].

Monitoring of pregnancy

Smoking during pregnancy reduces the mean body weight of neonates [49, 84] and increases the risk of delivering very small preterm infants [99]. The relationship between this reduced weight at birth and the increased risk of perinatal mortality, however, remains controversial. Several studies conclude that prenatal exposure to tobacco smoke is related to impaired mental development in infants [52, 62, 72, 86]; however, inconsistent results have been reported [84]. Finally, smoking during pregnancy could be associated with increased episodes of sudden death [11, 17] as well as with negative effects on lung function during the first few months of life [9, 31, 90]. It therefore seems essential to sensitize mothers to this health problem for their offspring and to assess their smoking status by an appropriate biochemistry test such as that of cotinine in urine [39, 49, 60, 64, 68, 85].

Smoking cessation programs

Urinary cotinine measurement may be very interesting as a motivation tool for smokers who are trying to stop. It allows not only assessment of the impact of smoking-cessation educational programs [92] but also augmentation of their efficacy [57]. However, this approach is valid only when nicotine substitution is not present (e.g., chewing gum, patch, nasal spray). In cases of nicotine substitution, other tobacco-smoke exposure biomarkers such as thiocyanate, carboxyhemoglobin, or carbon monoxide in expired air are suitable alternatives.

Monitoring of groups at risk

Besides its well-established (lung, upper respiratory airways, esophagus, and bladder) [35] or suspected (uterus) [88] carcinogenic effects, cigarette smoking is also a risk factor for cardiovascular disease. A correlation has been demonstrated between daily cigarette consumption as assessed by urinary cotinine determination and the urinary excretion of thromboxane A2 metabolites [71, 95, 98] but not with urinary excretion of prostacyclin metabolites. This observation can be explained by in vivo platelet activation [98]. As smoking habits, generally acquired at a very early age, are especially deleterious for the cardiovascular function of some groups at risk, including diabetics, it would therefore be useful to adopt for such groups early health education programs in which urinary cotinine determination may be used to assess smoking status [7, 48, 81, 87].

Epidemiological studies and assessment of occupational exposure to industrial pollutants

In epidemiological studies on exposure to occupational pollutants, it may be very important to distinguish smokers from nonsmokers. Evaluation of the toxic effects of occupational respiratory irritants requires the recognition of the smoking habits of exposed subjects; for this purpose, urinary cotinine determination may be very useful for the validation of information collected by questionnaire. Occupational exposure to carbon monoxide (CO) may be assessed by direct determination of

the gas itself either in environmental air or in the expired air of exposed workers. Another monitoring test consists of blood carboxyhemoglobin (HbCO) determination. For this use a tolerable level of blood HbCO (3.5%) has been proposed; it corresponds to a mean working-air CO exposure of 25 ppm for an 8-h work shift [43]. In smokers, blood HbCO determination alone overestimates CO occupational exposure intensity. Simultaneous determination of blood HbCO and urinary cotinine would allow evaluation of the interference factor related to smoking habits. In the same way, the establishment of biological limits in the framework of occupational exposure monitoring of organic substances may require analysis of the relationship between the ambient concentration of a parent substance (for which limit values may already exist) and the levels of its metabolites in biological media. As tobacco smoke contains many organic substances (e.g., benzene, polycyclic aromatic hydrocarbons (PAH)], reference values for metabolites, i.e., *trans,trans*-muconic acid for benzene and 1-hydroxypyrene for PAH, must take smoking habits into account as interference factors [54, 55, 66].

Phase I clinical trials

Before the introduction of a new drug, several studies are legally required both in animals and in humans. In the latter, clinical investigations called phase I trials use healthy volunteers. Since cigarette smoking may influence not only the pharmacokinetic profile but also the pharmacodynamic characteristics of numerous substances used in therapy (e.g., benzodiazepines, theophylline) [56], it may be necessary to verify the recent smoking history of selected individuals by the determination of a biological marker such as cotinine in urine, which seems more reliable in determining tobacco use than a simple questionnaire [1].

Control of life-insurance candidate declaration

Because of the number of risks related to active smoking, the life expectancy of a smoker is decreased. Insurance companies are therefore very interested in tests such as the measurement of cotinine in urine for establishment of the smoking status of candidates for life insurance. For economic (test costs) rather than strictly scientific reasons, insurance companies do not seek to obtain low detection limits but accept relatively important threshold values between smokers and nonsmokers (500 ng/ml) [1], which entails the likelihood of their missing some smokers who have abstained for only 1 day or less.

Conclusion

Cotinine remains the biomarker of choice for assessment of tobacco smoke exposure. Its urinary determination can be recommended in several situations such as monitoring of ETS exposure, impact evaluation of smoking cessation programs, monitoring of pregnancy and of some groups at risk, assessment of occupational exposure to industrial pollutants, validation of phase I clinical trials, and the control of life-insurance candidate declaration. Even if this review has focused on tobacco smoke exposure, it should be mentioned that urinary cotinine determination may also be used either as a marker of exposure to other tobacco products (nasal, "chique") or as a compliance index in nicotine substitution programs (chewing gum, patch, or nasal spray with nicotine). With regard to analytical aspects, only immunological and chromatography methods are sufficiently sensitive to assess exposure to ETS, and the choice between these two approaches could be influenced by the availability of the apparatus.

References

1. Apseloff G, Ashton HM, Friedman H, Gerber N (1994) The importance of measuring cotinine levels to identify smokers in clinical trials. Clin Pharmacol Ther 56: 460–462
2. Bakoula CG, Kafritsa YI, Kavadias GD, Lazopoulou DD, Theodoridou MC, Maravelias KP, Matsaniotis NS (1995) Objective passive-smoking indicators and respiratory morbidity in young children. Lancet 346: 280–281
3. Barlow RD, Thompson PA, Stone RB (1987) Simultaneous determination of nicotine, cotinine and five additional nicotine metabolites in the urine of smokers using pre-column derivatisation and high-performance liquid chromatography. J Chromatogr 419: 375–380
4. Benowitz NL (1996) Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev 18: 188–204
5. Benowitz NL, Kuyt F, Jacob P, Jones RT, Osman AL (1983) Cotinine disposition and effects. Clin Pharmacol Ther 34: 604–611
6. Biber A, Scherer G, Hoepfner I, Adlkofer F, Heller WD, Haddow JE, Knight GJ (1987) Determination of nicotine and cotinine in human serum and urine: an interlaboratory study. Toxicol Lett 35: 45–52
7. Bodmer CW, MacFarlane IA, Flavell HJ, Wallymahmed M, Calverley PM (1990) How accurate is the smoking history in newly diagnosed diabetic patients? Diabetes Res Clin Pract 10: 215–220
8. Bono R, Russo R, Arossa W, Scursatone E, Gilli G (1996) Involuntary exposure to tobacco smoke in adolescents: urinary cotinine and environmental factors. Arch Environ Health 51: 127–131
9. Brown RW, Hanrahan JP, Castile RG, Tager IB (1995) Effect of maternal smoking during pregnancy on passive respiratory mechanics in early infancy. Pediatr Pulmonol 19: 23–28
10. Bruckert E, Jacob N, Lamaire L, Truffert J, Percheron F, de-Gennes JL (1992) Relationship between smoking status and serum lipids in a hyperlipidemic population and analysis of possible confounding factors. Clin Chem 38: 1698–1705
11. Bulterys MG, Greenland S, Kraus JF (1990) Chronic fetal hypoxia and sudden infant death syndrome: interaction between maternal smoking and low hematocrit during pregnancy. Pediatrics 86: 535–540
12. Byrd GD, Chang KM, Greene JM, deBethizy JD (1992) Evidence for urinary excretion of glucuronide conjugates of nicotine, cotinine and trans-3'-hydroxycotinine in smokers. Drug Metab Dispos 20: 192–197
13. Casale R, Colantonio D, Cialente M, Colorizio V, Barnabei R, Pasqualetti P (1991) Impaired pulmonary function in schoolchildren exposed to passive smoking. Detection by

questionnaire and urinary cotinine levels. Respiration 58: 198–203
14. Chilmonczyk BA, Knight GJ, Palomaki GE, Pulkkinen AJ, Williams J, Haddow JE (1990) Environmental tobacco smoke exposure during infancy. Am J Public Health 80: 1205–1208
15. Chilmonczyk BA, Palomaki GE, Knight GJ, Williams J, Haddow JE (1992) An unsuccessful cotinine-assisted intervention strategy to reduce environmental tobacco smoke exposure during infancy. Am J Dis Child 146: 357–360
16. Chilmonczyk BA, Salmun LM, Megathlin KN, Neveux LM, Palomaki GE, Knight GJ, Pulkkinen AJ, Haddow JE (1993) Association between exposure to environmental tobacco smoke and exacerbations of asthma in children. N Engl J Med 328: 1665–1669
17. Cnattingius S, Haglund B, Meirik O (1988) Cigarette smoke as risk factor for late fetal and early neonatal death. BMJ 279: 258–261
18. Daenens P, Larueile L, Calleweart K, DeScheepar P, Galeazzi R, Van Rossum J (1985) Determination of cotinine in biological fluids by capillary gas chromatography-mass spectrometry selected ion monitoring. J Chromatogr 342: 79–87
19. Dahlstrom A, Lundell B, Curvall M, Thapper L (1990) Nicotine and cotinine concentrations in the nursing mother and her infant. Acta Paediatr Scand 79: 142–147
20. Davis RA, Stiles MF, deBethizy JD, Reynolds JH (1991) Dietary nicotine: a source of urinary cotinine. Food Chem Toxico 29: 821–827
21. Dell'Orco V, Forastiere F, Agabiti N, Corbo GM, Pistelli R, Pacifici R, Zuccaro P, Pizzabiocca A, Rosa M, Altieri I, et al (1995) Household and community determinants of exposure to involuntary smoking: a study of urinary cotinine in children and adolescents. Am J Epidemiol 142: 419–427
22. De Waard F, Kemmeren JM, Ginkel LA van, Stolker AA (1995) Urinary cotinine and lung cancer risk in a female cohort. Br J Cancer 72: 784–787
23. Domino EF (1995) Nonsmobacco sources of cotinine in the urine of nonsmokers. Clin Pharmacol Ther 57: 479
24. Ehrlich R, Kattan M, Godbold J, Salzberg DS, Grimm KT, Landrigan PJ, Lilienfeld DE (1992) Childhood asthma and passive smoking. Urinary cotinine as a biomarker of exposure. Am Rev Respir Dis 145: 594–599
25. Eremin SA, Coxon RE, Colbert DL, Landon J, Smith DS (1992) Urinary cotinine fluoroimmunoassay for smoking status screening adapted to an automated analyser. Analyst 117: 697–699
26. Feyerabend C, Russell MA (1990) A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. J Pharm Pharmacol 42: 450–452
27. Fried PA, Perkins SL, Watkinson B, McCartney JS (1995) Association between creatinine-adjusted and unadjusted urine cotinine values in children and the mother's report of exposure to environmental tobacco smoke. Clin Biochem 28: 415–420
28. Gaffari E, Marunelli G, Romeo L, Durigato S (1992) Esposizione al fumo di tabacco degli impiegati di un'azienda telefonica. Med Lav 83: 596–604
29. Galanti LM, Manigart P, Dubois P (1995) Inverse relationship between tobacco smoking and both psychotechnic and education levels. Arch Environ Health 50: 381–383
30. Greenberg RA, Bauman KE, Strecher VJ, Keyes LL, Glover LH, Haley NJ, Stedman HC, Loda FA (1991) Passive smoking during the first year of life. Am J Public Health 81: 850–853
31. Hanrahan JP, Tager IB, Segal MR, Tosteson TD, Castile RG, Van Vunakis H, Weiss ST, Speizer FE (1992) The effect of maternal smoking during pregnancy on early infant lung function. Am Rev Respir Dis 145: 1129–1135
32. Hariharan M, VanNoord T (1991) Liquid-chromatographic determination of nicotine and cotinine in urine from passive smokers: comparison with gas chromatography with nitrogen-specific detector. Clin Chem 37: 1276–1280
33. Horsmann M (1985) Simple high-performance liquid chromatographic method for rapid determination of nicotine and cotinine in urine. J Chromatogr 344: 391–396
34. Idle JR (1990) Titrating exposure to tobacco smoke using cotinine – a minefield of misunderstandings. J Clin Epidemiol 43: 313–317
35. International Agency for Research on Cancer (1986) Tobacco smoking. IARC Monogr Eval Carcinog Risk Chem Hum 38: 270
36. Jacob P, Shulgin AT, Yu L, Benowitz NL (1992) Determination of the nicotine metabolite trans-3'-hydroxycotinine in urine of smokers using gas chromatography with nitrogen-selective detection or selected ion monitoring. J Chromatogr 583: 145–154
37. Jacob P, Yu L, Liang G, Shulgin AT, Benowitz NL (1993) Gas chromatographic-mass spectrometric method for determination of anabasine, anatabine and other tobacco alkaloids in urine of smokers and smokeless tobacco users. J Chromatogr 619: 49–61
38. Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, Vesey C, Saloojee Y (1987) Comparison of tests used to distinguish smokers from nonsmokers. Am J Public Health 77: 1435–1438
39. Kendrick JS, Zahniser SC, Miller N, Salas N, Stine J, Gargiullo PM, Floyd RL, Spierto FW, Sexton M, Metzger RW et al (1995) Integrating smoking cessation into routine public prenatal care: the Smoking Cessation in Pregnancy project. Am J Public Health 85: 217–222
40. Knight JM, Eliopoulos C, Klein J, Greenwald M, Koren G (1996) Passive smoking in children. Racial differences in systemic exposure to cotinine by hair and urine analysis. Chest 109: 446–450
41. Kolonen SA, Puhakainen EV (1991) Assessment of the automated colorimetric and the high-performance liquid chromatographic methods for nicotine intake by urine samples of smokers smoking low- and medium-yield cigarettes. Clin Chim Acta 196: 159–166
42. Laurent AM, Bevan A, Chakroun N, Courtois Y, Valois B, Roussel M, Festy B, Pretet S (1992) Effets sanitaires d'une exposition chronique à la fumée de tabac sur une population de non fumeurs. Rev Pneumol Clin 48: 65–70
43. Lauwerys RR, Hoet P (1993) Industrial chemical exposure: guidelines for biological monitoring. Lewis, Boca Raton, Florida
44. Langone JJ, Bjercke RJ (1989) Idiotype–anti-idiotype hapten immunoassays: assay for cotinine. Anal Biochem 182: 187–192
45. Langone JJ, Cook G, Bjercke RJ, Lifschitz MH (1988) Monoclonal antibody ELISA for cotinine in the urine of active and passive smokers. J Immunol Methods 114: 73–78
46. Lee PN (1995) "Marriage to a smoker" may not be a valid marker of exposure in studies relating environmental tobacco smoke to risk of lung cancer in Japanese non-smoking women. Int Arch Occup Environ Health 67: 287–294
47. Marbury MC, Hammond SK, Haley NJ (1993) Measuring exposure to environmental tobacco smoke in studies of acute health effects. Am J Epidemiol 137: 1089–1097
48. Masson EA, MacFarlane IA, Priestley CJ, Wallymahmed ME, Flavell HJ (1992) Failure to prevent nicotine addition in young people with diabetes. Arch Dis Child 67: 100–102
49. Mathai M, Skinner A, Lawton K, Weindling AM (1990) Maternal smoking, urinary cotinine levels and birth-weight. Aust N Z J Obstet Gynaecol 30: 33–36
50. Matsushita M, Shionoya S, Matsumoto T (1991) Urinary cotinine measurement in patients with Buerger's disease: effects of active and passive smoking on the disease process. J Vasc Surg 14: 53–58
51. McAdams SA, Cordeiro ML (1993) Simple selected ion monitoring capillary gas chromatographic-mass spectrometric method for the determination of cotinine in serum, urine and oral samples. J Chromatogr 615: 148–153
52. McCartney JS, Fried PA, Watkinson B (1994) Central auditory processing in school-age children prenatally exposed to cigarette smoke. Neurotoxicol Teratol 16: 269–276

53. McManus KT, deBethizy JD, Garteiz DA, Kyerematen GA, Vesell ES (1990) A new quantitative thermospray LC-MS method for nicotine and its metabolites in biological fluids. J Chromatogr Sci 28: 510–516
54. Melikian AA, Prahalad AK, Hoffmann D (1993) Urinary *trans,trans*-muconic acid as an indicator of exposure to benzene in cigarette smokers. Cancer Epidemiol Biomarkers Prev 2: 47–51
55. Melikian AA, Prahalad AK, Secker-Walker RH (1994) Comparison of the levels of the urinary benzene metabolite *trans,trans*-muconic acid in smokers and nonsmokers, and the effects of pregnancy. Cancer Epidemiol Biomarkers Prev 3: 239–244
56. Miller LG (1989) Recent developments in the study of the effects of cigarette smoking on clinical pharmacokinetics and clinical pharmacodynamics. Clin Pharmacokinet 17: 90–108
57. Miwa K, Miyagi Y, Asanoi H, Fujita M, Sasayama S (1993) Augmentation of smoking cessation education by urinary cotinine measurement. Jpn Circ J 57: 775–780
58. Miwa K, Fujita M, Miyagi Y (1994) Beneficial effects of smoking cessation on the short term prognosis for variant angina–validation of the smoking status by urinary cotinine measurements. Int J Cardiol 44: 151–156
59. Moore J, Greenwood M, Sinclair N (1990) Automation of a high-performance liquid chromatographic assay for the determination of nicotine, cotinine and 3-hydroxycotinine in human urine. J Pharm Biomed Anal 8: 1051–1054
60. Mullen PD, Carbonari JP, Tabak ER, Glenday MC (1991) Improving disclosure of smoking by pregnant women. Am J Obstet Gynecol 165: 409–413
61. Murray A, Morrison B (1986) The effect of cigarette smoke from the mother on bronchial responsiveness and severity of symptoms in children with asthma. J Allergy Clin Immunol 77: 575–581
62. Naeye RL, Peters EC (1984) Mental development of children whose mothers smoked during pregnancy and effects on the fetus. Obstet Gynecol 64: 601–607
63. Neurath GB, Dünger M, Orth D, Pein FG (1987) Trans-3'-hydroxycotinine as a main metabolite in urine of smokers. Int Arch Occup Environ Health 59: 199–201
64. O'Connor AM, Davies BL, Dulberg CS, Buhler PL, Nadon C, McBride BH, Bendle RJ (1992) Effectiveness of a pregnancy smoking cessation program. J Obstet Gynecol Neonatal Nurs 21: 385–392
65. Oldigs M, Jorres R, Magnussen H (1991) Acute effect of passive smoking on lung function and airway responsiveness in asthmatic children. Pediatr Pulmonol 10: 123–131
66. Ong CN, Lee BL, Shi CY, Ong HY, Lee HP (1994) Elevated levels of benzene-related compounds in the urine of cigarette smokers. Int J Cancer 59: 177–180
67. Parviainen MT, Barlow RD (1988) Assessment of exposure to environmental tobacco smoke using a high-performance liquid chromatographic method for the simultaneous determination of nicotine and two of its metabolites in urine. J Chromatogr 431: 216–221
68. Petersen L, Handel J, Kotch J, Podedworny T, Rosen A (1992) Smoking reduction during pregnancy by a program of self-help and clinical support. Obstet Gynecol 79: 924–930
69. Phillipou G, James SK, Seaborn CJ, Phillips PJ (1994) Assessment of a simple colorimetric procedure to determine smoking status in diabetic subjects. Clin Chem 40: 1296–1298
70. Pickert A, Lingenfelser T, Pickert C, Birbaumer N, Overkamp D, Eggstein M (1993) Comparison of a mechanized version of the "König" reaction and a fluorescence polarization immunoassay for the determination of nicotine metabolites in urine. Clin Chim Acta 217: 143–152
71. Rangemark C, Ciabattoni G, Wennmalm A (1993) Excretion of thromboxane metabolites in healthy women after cessation of smoking. Arterioscler Thromb 13: 777–782
72. Rantakallio P (1983) A follow-up to the age of 14 of children whose mothers smoked during pregnancy. Acta Paediatr Scand 72: 747–753
73. Reese AC, James IR, Landau LI, Lesouef PN (1992) Relationship between urinary cotinine level and diagnosis in children admitted to hospital. Am Rev Respir Dis 146: 66–70
74. Repace JL, Lowrey AH (1993) An enforceable indoor air quality standard for environmental tobacco smoke in the workplace. Risk Anal 13: 463–475
75. Riboli E, Preston-Martin S, Saracci R, Haley NJ, Trichopoulos D, Becher H, Burch JD, Fontham ET, Gao YT, Jindal SK (1990) Exposure of nonsmoking women to environmental tobacco smoke: a ten country collaborative study. Cancer Causes Control 1: 243–252
76. Riboli E, Haley NJ, Tredaniel J, Saracci R, Preston-Martin S, Trichopoulos D (1995) Misclassification of smoking status among women in relation to exposure to environmental tobacco smoke. Eur Respir J 8: 285–290
77. Rop PP, Grimaldi F, Oddoze C, Viala A (1993) Determination of nicotine and its main metabolites in urine by high-performance liquid chromatography. J Chromatogr 612: 302–309
78. Rustemeier K, Demetriou D, Schepers G, Voncken P (1993) High-performance liquid chromatographic determination of nicotine and its urinary metabolites via their 1,3-diethyl-2-thiobarbituric acid derivatives. J Chromatogr 613: 95–103
79. Rylander E, Pershagen G, Eriksson M, Bermann G (1995) Parental smoking, urinary cotinine, and wheezing bronchitis in children. Epidemiology 6: 289–293
80. Sampson EJ, Needham LL, Pirkle JL, Hannon WH, Miller DT, Patterson DG, Bernert JT, Ashley DL, Hill RH, Gunter BW, Paschal DC, Spierto FW, Rich MJ (1994) Technical and scientific developments in exposure marker methodology. Clin Chem 40: 1376–1384
81. Sawicki PT, Didjurgeit U, Muhlhauser I, Berger M (1993) Behaviour therapy versus doctor's anti-smoking advice in diabetic patients. J Intern Med 234: 407–409
82. Schepers G, Walk RA (1988) Cotinine determination by immunoassays may be influenced by other nicotine metabolites. Arch Toxicol 62: 395–397
83. Schneider JM, Capolaghi B, Briancon S, Covi G, Merlin JP, Leveau PH (1993) Le tabagisme passif de l'enfant. Son dépistage par le dosage de la cotinine urinaire. Arch Fr Pediatr 50: 567–571
84. Schulte-Hobein B, Schwartz-Bickenbach D, Abt S, Plum C, Nau H (1992) Cigarette smoke exposure and development of infants throughout the first year of life: influence of passive smoking and nursing on cotinine levels in breast milk and infant's urine. Acta Paediatr verified 81: 550–557
85. Secker-Walker RH, Solomon LJ, Flynn BS, Skelly JM, Lepage SS, Goodwin GD, Mead PB (1995) Smoking relapse prevention counseling during prenatal and early postnatal care. Am J Prev Med 11: 86–93
86. Sexton M, Fox NL, Hebel JR (1990) Prenatal exposure to tobacco. II. Effects on cognitive functioning at age three. Int J Epidemiol 19: 72–77
87. Shaw NJ, McClure RJ, Kerr S, Lawton K, Smith CS (1993) Smoking in diabetic teenagers. Diabet Med 10: 275–277
88. Simons AM, Phillips DH, Coleman DV (1993) Damage to DNA in cervical epithelium related to smoking tobacco. BMJ 306: 1444–1448
89. Skarping G, Willers S, Dalene M (1988) Determination of cotinine in urine using glass capillary gas chromatography and selective detection, with special reference to the biological monitoring of passive smoking. J Chromatogr 454: 293–301
90. Tager IB, Ngo L, Hanrahan JP (1995) Maternal smoking during pregnancy. Effects on lung function during the first 18 months of life. Am J Respir Crit Care Med 152: 977–983
91. Thompson SG, Stone R, Nanchahal K, Wald NJ (1990) Relation of urinary cotinine concentrations to cigarette smoking and to exposure to other people's smoke. Thorax 45: 356–361

92. Trudeau DL, Isenhart C, Silversmith D (1995) Efficacy of smoking cessation strategies in a treatment program. J Addict Dis 14: 109–116
93. Tweedie RL, Mengersen KL (1992) Lung cancer and passive smoking: reconciling the biochemical and epidemiological approaches. Br J Cancer 66: 700–705
94. Ubbink JB, Lagendijk J, Vermaak WH (1993) Simple high-performance chromatographic method to verify the direct barbituric acid assay for urinary cotinine. J Chromatogr 620: 254–259
95. Uerdelhoven WM, Rutzel A, Meese CO, Weber PC (1991) Smoking alters thromboxane metabolism in man. Biochim Biophys Acta 1081: 197–201
96. Vine MF, Hulka BS, Margolin BH, Truong YK, Hu PC, Schramm MM, Griffith JD, McCann M, Everson RB (1993) Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. Am J Public Health 83: 1335–1338
97. Watts RR, Langone JJ, Knight GJ, Lewtas J (1990) Cotinine analytical workshop report: consideration of analytical methods for determining cotinine in human body fluids as a measure of passive exposure to tobacco smoke. Environ Health Perspect 84: 173–182
98. Wennmalm A, Benthin G, Granstrom EF, Persson L, Petersson AS, Winell S (1991) Relation between tobacco use and urinary excretion of thromboxane A2 and prostacyclin metabolites in young men. Circulation 83: 1698–1704
99. Wilcox AJ (1993) Birth weight and perinatal mortality: the effect of maternal smoking. Am J Epidemiol 137: 1098–1104
100. Willers S, Svenonius E, Skarping G (1991) Passive smoking and childhood asthma. Urinary cotinine levels in children with asthma and in referents. Allergy 46: 330–334
101. Willers S, Attewell R, Bonaryd I, Schutz A, Skarping G, Vgbier M (1992) Exposure to environmental tobacco smoke in the household and urinary cotinine excretion, heavy metals retention, and lung function. Arch Environ Health 47: 357–363
102. Willers S, Skarping G, Dalene M, Skerfving S (1995) Urinary cotinine in children and adults during and after semiexperimental exposure to environmental tobacco smoke. Arch Environ Health 50: 130–138
103. Zuccaro P, Altieri I, Rosa M, Passa AR, Pichini S, Pacifici R (1995) Solid-phase extraction of nicotine and its metabolites for high-performance liquid chromatographic determination in urine. J Chromatogr 668 B: 187–188